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Award Number: W81XWH-06-1-0151

TITLE: Epigenetic Regulation of Chemokine Expression in Prostate Cancer

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REPORT DATE: December 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-12-2007	Annual	1 DEC 2006 - 30 NOV 2007

4. TITLE AND SUBTITLE

Epigenetic Regulation of Chemokine Expression in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0151

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pittsburgh
Pittsburgh, PA 15213

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
No abstract provided.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	8	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Introduction

Chemokines play an important role in initiating immune responses by regulating attraction and homing of immune cells to the lymphoid tissues. Breast and kidney-expressed chemokine (BRAK; CXCL14) is known to be selective for monocytes and dendritic cells (DC). CXCL14 is expressed ubiquitously in normal tissues, but, as has been recently shown, absent in a variety of cancer tissues and tumor cell lines. However, the mechanisms responsible for CXCL14 loss in malignant tissues and cells are unknown. The main goal of this proposal is to determine the mechanisms of the regulation of CXCL14 expression by prostate cancer and test whether recovery of CXCL14 expression on tumor cells will be accomplished by attraction of DC and boosting of antitumor immune responses. During the first year of support, we evaluated chemoattractive properties of CXCL14 towards DC and demonstrated that human prostate cancer tissues and prostate cancer cell lines LNCaP, PC3, and DU145 do not express CXCL14 protein and mRNA and do not chemoattract DC in vitro and in vivo. The focus of Task 2 was to determine whether the restoration of CXCL14 expression on tumor cells might be associated with DC attraction in vitro and in vivo. Our data are briefly summarized below.

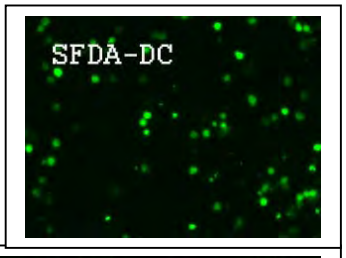
Body

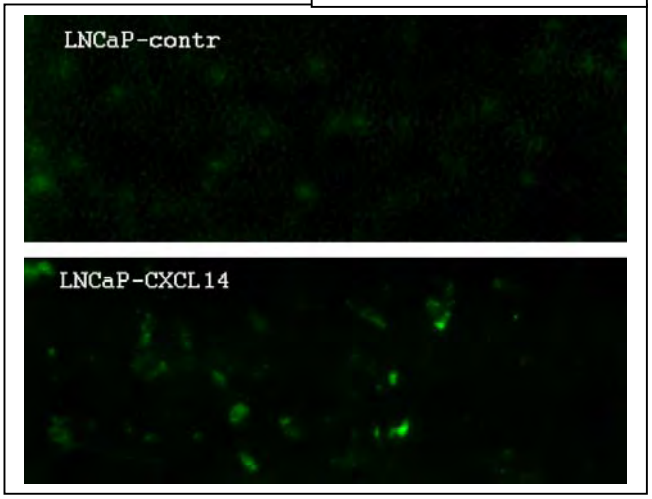
a. Functional analysis of different DC subtypes in chemotaxis assay.

We finalized Task 1 by comparing chemotaxis of DC generated in vitro from different precursor cells. All studies carried out during Year 1 (Task 1) utilized DC generated from CD14+ monocyte precursors cultured with GM-CSF and IL-4. Here, we prepared DC from CD34+ hematopoietic precursors generated with a cocktail of cytokines and growth factors as we described earlier [Shurin, 2003 #1]. DC attraction by CXCL14 was assessed in a Transwell system as we described earlier [Shurin, 2005 #2]. The results of these studies revealed that CD34-derived DC are attracted by CXCL14 in vitro and no differences between CD14-derived and CD34-derived DC in terms of their migration towards CXCL14 were noticed. Thus, both monocyte-derived DC and hematopoietic cell-derived DC express CXCL14 receptors and might be attracted by this chemokine in vitro.

b. Analysis of DC migration towards CXCL14-positive and negative prostate cancer cells growing in vivo

The goal of these studies was to compare and contrast the ability of CXCL14-expressing and CXCL14-negative prostate cancer cells to chemoattract human DC in vivo. Based on the results of Task 1, LNCaP cell line was chosen and CXCL14-negative cell line, which did not attract DC in vitro in standard conditions. Usinf immunocompromized SCID mice, we first demonstrated that

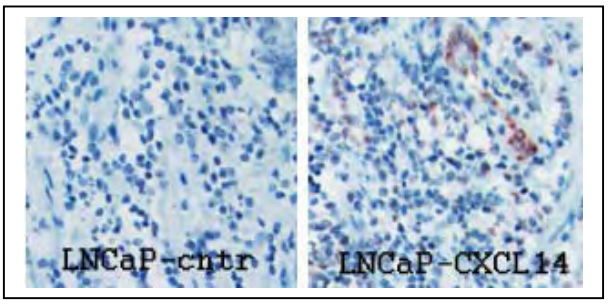




CXCL14 cells growing in vivo did not chemoattract human DC that we generated ex vivo from CD14-precursors, labeled with green fluorescent dye PKH and injected intravenously in tumor-bearing mice (5-10 x 10^6cells/0.5 ml HBCC) 2 weeks after tumor cell inoculation or when tumor reached 30-50 mm^2 size. 24,48 and 72 hours later, tumor mass was harvested, frozen, and cutted for the confocal microscopy evaluation. The results of two independent experiments revealed no significant migration of exogenous DC to the tumor site. However, if LNCaP cells were forced to secrete CXCL14 when growing in SCID mice prior to

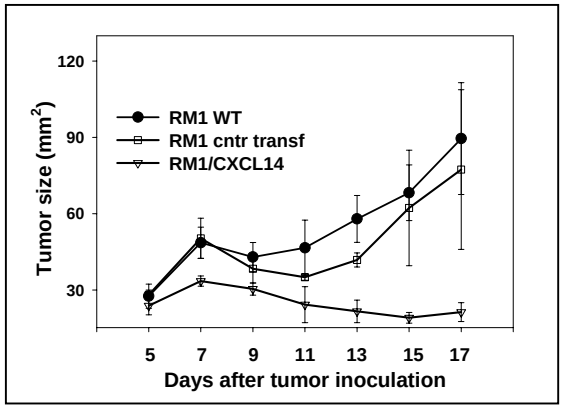
administration of fluorescent-labeled human DC, homing of DC at the tumor site was visible and significant in comparison to CXCL14-negative tumors. These results suggest that CXCL14 chemokine might serve as DC chemoattractant in vivo. Another conclusion was that loss of CXCL14 expression by prostate cancer cells might serve as a mechanism of immune escape.

c. Analysis of human DC in human prostate carcinomas in SCID mice by immunohistochemistry

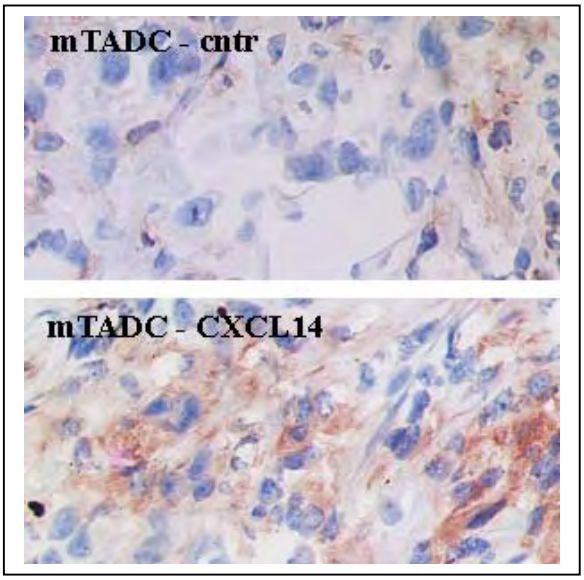


Conformation of chemoattractive role of prostate cancer-derived CXCL14 for human DC in vivo was obtained by visualizing DC in the tumor section by immunohistochemistry. DC staining was done as described earlier using CD11c and CD83 antibodies (Perez et al., 2005). As expected, expression of CXCL14 in tumor cells in vivo was associated with increased homing of DC at the tumor bed. Interestingly, our preliminary data suggest that the areas of accumulation of tumor-associated DC (TADC) corresponded to the areas of CXCL14 expression inside of the tumor mass. This attractive observation requires further confirmation and verification and this analysis is in progress in the lab in collaboration with experienced pathologist. Thus, altogether, the results of Task 2a studies support our main hypothesis that CXCL14 is an important DC attracting chemokine whose expression is lost in malignant prostate tissues. Replacement of CXCL14 expression in prostate cancer cells resulted in attraction and accumulation of TADC. The next question was whether this replacement might have any therapeutic benefit.

d. Analyze the therapeutic potential of CXCL14 expression in prostate cancer

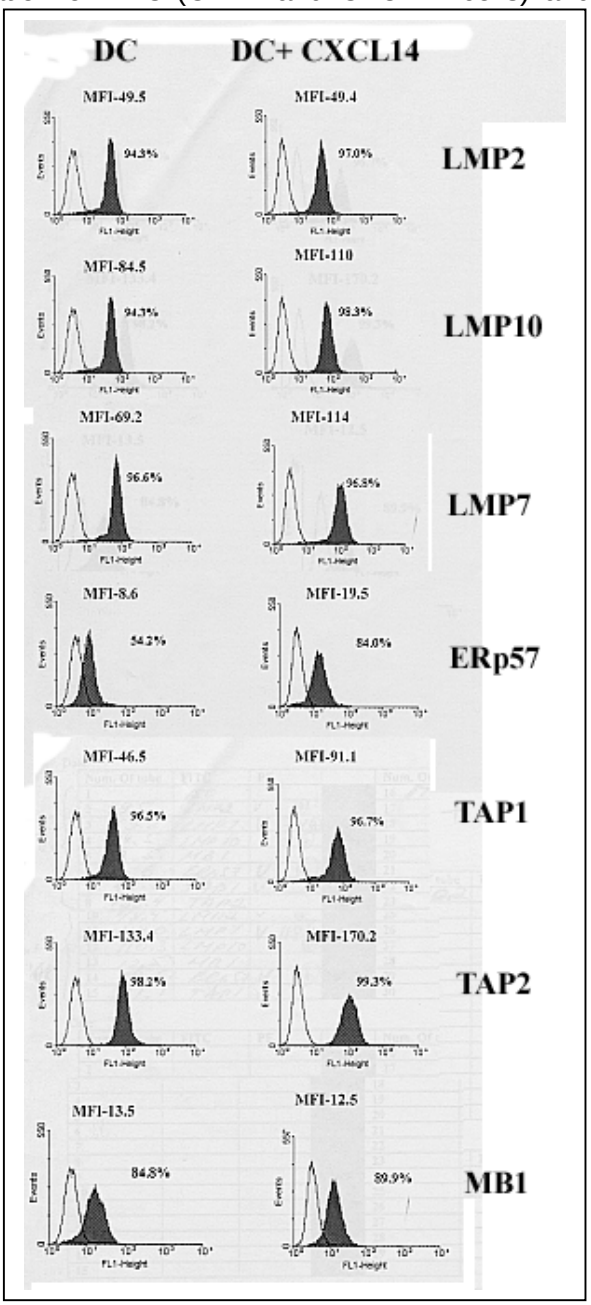


We next tested if transfection of murine prostate cancer cell lines with the CXCL14 gene alter tumor growth in immunocompetent syngeneic mice. Initially two murine cell lines were used for these studies – RM1 and TRAMP-C2. Both cell lines were transduced with the CXCL14 gene or control empty vector and cells were cultured for selection as we described in the original application for other murine cell lines. CXCL14 positive and negative RM1 cell lines were obtained as expected. However, very slow growing TRAMP-C2 cells were not selected after three attempts due to the very low expression of CXCL14 protein, as determined by Western blot. This might be explained by their low growth rate or some additional unknown features. The results obtained with RM1 cells revealed that enforced expression of the chemokine in tumor cells was associated with marked and statistically significant inhibition of tumor growth in vivo. An important control included the analysis of treated and intact tumor cell line growth in vitro to rule out the possibility of direct inhibition of tumor longevity by CXCL14. No differences in tumor cell proliferation in vitro, assessed by MTT assay, were determined. Thus, these data suggest that CXCL14 has a potential antitumor activity in vivo.



Next important question was whether CXCL14-

mediated antitumor effect was linked to the induction of antitumor immunity. To test this, we first harvested all tumor tissues and analyzed them for the presence of tumor infiltrating leukocytes, since the appearance of these cells at the tumor site was shown to be associated with antitumor immune responses. Immunohistochemical evaluation of TILs (CD4+ and CD8+ T cells) and tumor-associated dendritic cells (TADC) revealed higher levels of DC and T cells, especially CD8+ lymphocytes, in CCL14-expressing tissues when compared to intact or control-transduced tumors. These data suggest that CXCL14 may attract DC to the tumor site, where DC can engulf tumor antigen, process it, migrate to the regional lymph nodes, present tumor antigen to antigen-specific T cells and, thus, induce antitumor immune responses. Together with additional data showing the formation of tumor-specific T cells in lymph nodes of mice inoculated with CXCL14-positive tumor cells, our results support our hypothesis about important role of DC chemokine CXCL14 in immunosurveillance.



Based on the above information, we speculated that CXCL14 might also potentiate antigen-presenting ability of DC. To obtain more important translational data, this hypothesis was tested using human DC generated from CD14+ precursor cells. In these studies, we evaluated ability of CXCL14 to alter expression of antigen-processing machinery (APM) components in DC, which correlate with antigen-presenting function of DC. APM components in DC were assessed by flow cytometry as we described earlier (Tourkova et al., 2005). Immature DC were treated with medium alone (control) or CXCL14 for 24 hours, harvested, washed and stained for different components of APM pathways, including proteasome, chaperon, and TAP proteins. The results were expressed as the percentage of cells expressing a protein and mean fluorescence intensity (MFI) reflecting the level of expression. Our data demonstrated that CXCL14 was able to slightly up-regulate the percentage of positive cells with the exception of Erp57, where the percentage of positive DC was doubled. Furthermore CXCL14 significantly increase the MFI values for many APM components, suggesting its unique role in up-regulating antigen presenting capacity of DC.

In summary, these and other results of Task 2 demonstrated that restoration of CXCL14 expression in prostate cancer cells was associated with increased attraction of DC both in vitro and in vivo, which, in turn, was accomplished by induction of antitumor immune responses in vivo. The next key question, which will be investigated in Task 3, is the mechanism responsible for low or no expression of CXCL14 chemokine in prostate cancer cells.

Key Research Accomplishments

- Prostate carcinoma cells that loss expression of CXCL14 chemokine do not attract DC in vivo in the chimeric tumor model
- Induced expression of CXCL14 in prostate cancer cell lines recovered their ability to chemoattract DC in vivo in the chimeric mouse model
- Chemoattraction of exogenous fluorescent-labeled DC to CXCL14-positive tumor mass growing in mice was determined by confocal microscopy and confirmed by immunohistochemistry
- Growth of CXCL14-positive prostate cancer cells in vivo in immunocompetent animals was significantly slower that growth of CXCL14-negative tumor cells
- CXCL14-positive prostate carcinomas growing in vivo were infiltrated by DC and T lymphocytes
- Inhibited growth of CXCL14-positive tumor cells was associated with the induction of local and systemic antitumor immunity in mice
- CXCL14 chemokine is not only a potent chemoattractant for DC, but it also a potent inducer of expression of antigen processing machinery components in DC.

Reportable Outcomes

PUBLICATIONS:

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PRESENTATIONS:

- Shurin M.R. “Mechanisms and therapeutic reversal of dendritic cell dysfunction in cancer”. Molecular Targets in Cancer Therapy: Fourth Biennial Meeting “Mechanism and Therapeutic Reversal of Immune Suppression in Cancer”, Clearwater Beach, FL. January 2007.
- Shurin M.R. “Dendritic cells in the tumor microenvironment: from experiments to therapy”. The 4th Intl Conference on Tumor Microenvironment: Progression, Therapy and Prevention. Florence, Italy. March 2007.
- Shurin M.R. “Dendritic cell-based cancer vaccines”. Chest Hospital, Shanghai, China, May 2007.
- Shurin M.R. “Regulation of the dendritic cell system in cancer”. Inst. for Immunology, 2nd Medical Military Academy, Shanghai, China. May 2007.
- Shurin M.R. “How do dendritic cells mediate immune escape in cancer?” 2nd Intl. Immune-Mediated Diseases Congress, Moscow, Russia. September 2007.
- Shurin M.R., Shurin G.V., Gutkin D.W**.** Regulation of CXCL14 Expression and Dendritic Cell Attraction in Prostate Cancer. DoD IMPaCT meeting, Atlanta, GA, September 2007.

Conclusions

During the second year of support, we developed an additional progress toward the main goal of our proposal – understanding the mechanisms of chemokine regulation in prostate cancer. Specifically, we revealed the role of CXCL14 chemokine in regulating DC attraction and homing in prostate cancer tissue in vivo. We demonstrated that neither murine nor human DC migrate towards CXCL14-negative tumors in vivo, but could be attracted by induced expression of CXCL14 in tumor cells. Migration of DC to the tumor site was associated with increased tumor mass infiltration by T cells and inhibition of tumor growth in syngeneic murine tumor models.

References

Appendices